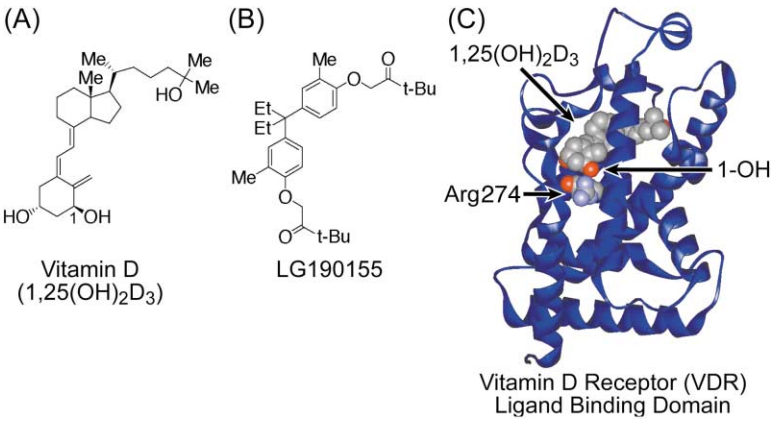

Natural and Nonnatural Vitamin Ds Compared with the Protein-Bound Vitamin

Structures of the active vitamin D metabolite $1,25(OH)_2D_3$ (A), the related synthetic agonist LG190155 (B), and the $1,25(OH)_2D_3$-VDR complex (C). In (C), the VDR arginine residue (Arg274) known to be mutated to a smaller hydrophobic leucine residue in cases of vitamin D-resistant rickets and the proximal 1-hydroxyl group (1-OH) of $1,25(OH)_2D_3$ modified to compensate for this mutation are explicitly shown.

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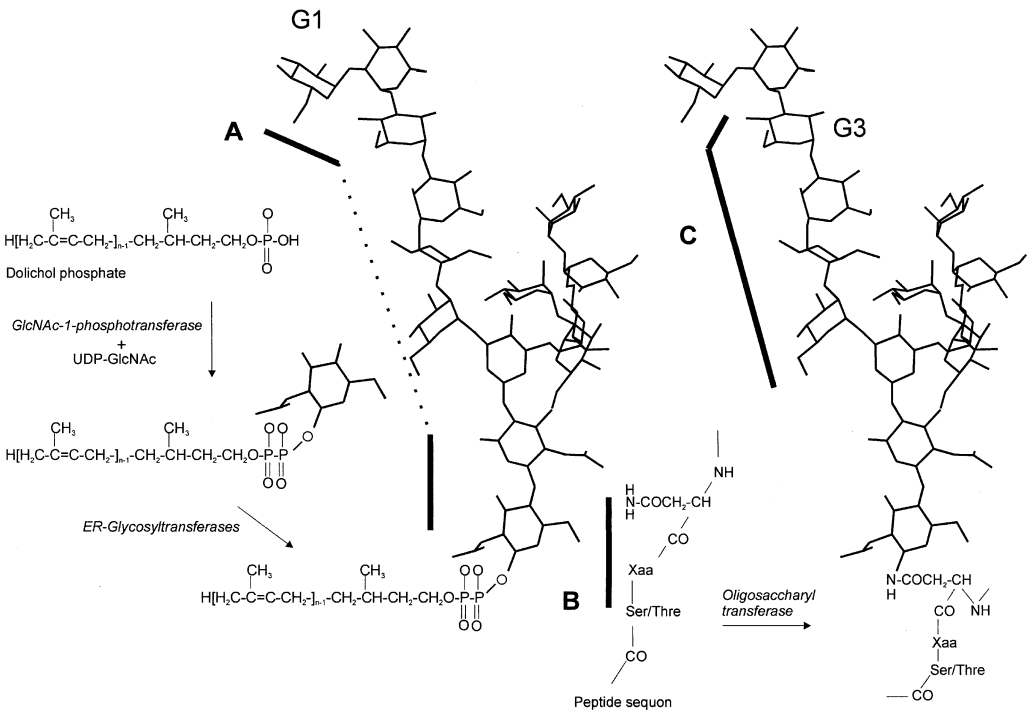
Control in the N-linked Glycoprotein Biosynthesis Pathway

N-linked glycosylation is vital for the development and maintenance of eukaryotic cells. The individual steps of this complex process are slowly being elucidated. In this issue, the Imperiali group further dissects the mechanics of oligosaccharyl transferase using substrate analogs.

The glycosylation of proteins requires a significant commitment of genetic and energetic resources by eukaryotic cells. Since this complex process was discovered, researchers have been trying to uncover and understand the various steps and molecular mechanisms of glycosylation that nature accomplishes with apparent ease. The critical importance of N-linked (or asparagine-linked) glycosylation to multicellular organization was demonstrated several years ago by Lennarz, who treated sea urchin embryos with tunicamycin to completely ablate N-linked glycosylation and observed the cessation of embryonic development [1]. Since that time, advances in gene manipulation have allowed researchers to conduct more elegant experiments to dissect these processes at the molecular level, and ultimately lend support to the hypotheses generated by Lennarz's earlier work. Marth and colleagues strived to disrupt the key glycosyltransferase genes using homologous recombination in mouse, and in this way showed that deletion of these genes resulted in embryonic lethality; interestingly, the earlier in the pathway the gene is deleted, the less progressive the embryonic development. Specifically, in this study, Marth and coworkers demonstrated that disrupting the gene that encodes for the dolichol GlcNAc-1-phosphotransferase (see figure), the enzyme inhibited by tunicamycin, blocks mouse development at gastrulation, precisely as Lennarz had shown earlier in sea urchins [2, 3]. By contrast, mouse embryos that were homozygous for a disrupted gene encoding the protein N-acetylglucosylaminyltransferase I, an enzyme that acts at a later stage in the glycosylation pathway for the synthesis of complex N-linked oligosaccharides, progress through the initial developmental events, but stop abruptly at embryonic stage E9.5–E10.5 [4, 5].

Intervening in the early steps in the dolichol lipid pathway for N-linked glycosylation by either chemical or



Oligosaccharide Transfer to Peptide in the Endoplasmic Reticulum

GlcNAc-1-phosphotransferase catalyses the first step in dolichol lipid oligosaccharide assembly. This step is inhibited by tunicamycin, preventing further N-linked glycosylation via a number of ER-resident glycosyltransferases that generate dolichol-P-P-$GlcNAc_2Man_9Glc_3$ as shown. The structure of the glycan is a molecular model based on the NMR structure (Petrescu et al.). The probable recognition elements that are required for oligosaccharyl transferase (OT) are shown. (A), an extended structure that includes the terminal α1,2 glucose residue (G1) and chitobiose core; (B), the Asn-Xaa-Ser/Thre motif. Following transfer, rearrangements of the peptide regions flanking the sequon stabilize the structure, resulting in weaker interaction at the active site. Processing of the protein-linked oligosaccharide by α-glucosidases I and II leaves a terminal α1,3 glucose residue (G3) that forms part of the calnexin binding motif (C).

biological means therefore has dramatic consequences for the cell. Some understanding of this effect has been gained by elucidating the role of carbohydrate ligands in critical lectin-like chaperone-mediated protein folding events in the endoplasmic reticulum (ER) [6]. Following transfer of the glycan from the dolichol lipid donor to the nascent protein, the glucose "cap" is sequentially removed from the now N-linked glycan by specific glucosidases in the ER lumen to leave a single glucose residue and several "exposed" mannose sugars. This glucose is a ligand recognized by calnexin to retain the nascent protein in the ER and allow the protein folding machinery, in particular, disulphide bond formation, to take place. If the deglucosylated protein is folded successfully, the glycan is further processed in the ER and Golgi to generate a mature glycoprotein. Another ER resident enzyme, a glucosyltransferase, detects misfolded proteins and directs calnexin to these proteins to assist with correct folding by transferring a single glucose residue to their glycan to be recognized by calnexin. This cycle continues until the protein has either adopted its folded conformation or is removed from the lumen and targeted to the lysosome for degradation. These molecular events have been identified in several eukaryotic species throughout the evolutionary hierarchy, suggesting that N-linked glycosylation may have been conserved to ensure the integrity of protein folding. Conversely, researchers have exploited this idea by using enzyme inhibitors that prevent monoglucosylated glycans from interacting with calnexin to promote incorrect protein folding in viral glycoproteins, which are glycosylated using the host cell pathway, as a potential treatment for many viral diseases, including hepatitis [7].

Key to eukaryotic N-linked glycosylation is the ribosomal complex, or translocon, that includes the oligosaccharyl transferase (OT) enzyme that transfers the assembled donor oligosaccharide from dolichol in the ER membrane to specific asparagine residues in the protein (see figure). Many of the structural and enzyme components of the translocon show a remarkable degree of homology in yeast and man, supporting the hypothesis that this process developed during early evolution. However, little structural information has been obtained for OT, and while we wait for the crystal structure of this enzyme, much can be gleaned from careful analysis of the substrate requirements of OT and by the use of site directed inhibitors of enzyme activity [8]. One interesting biological conundrum is how OT successfully negotiates product inhibition, since it must be able to accommodate the oligosaccharide attached to both lipid (dolichol) and the nascent protein. The OT may appear to be unregulated in many cells because the continuous rate of synthesis of the dolichol lipid precursor is not always equaled by the rate at which the oligosaccharide is transferred to the protein. Excess dolichyl oligosaccharide can be created when there are either insufficient

protein acceptors passing through the ER, or under circumstances where the N-glycosylation consensus sequence (Asn-X-Ser/Thre motif) is not glycosylated. Although this site is consistently glycosylated in many individual proteins, the local protein environment may dictate incomplete glycosylation. Currently, a clear understanding of this mechanism is lacking, but in either case, the resultant dolichyl oligosaccharide is cleaved and free oligosaccharide is generated. This oligosaccharide is eventually cleared from the lumen of the ER to the lysosome, after limited digestion with cytosolic mannosidases [9].

In this issue, Imperiali's group have attempted to rationalize the mechanism that allows the constitutive rate of expression of OT to be regulated at the level of substrate interactions with the active site [10]. Using neoglycopeptides, the authors propose that the oligosaccharide is transferred to the peptide, in a *cis*-amide conformation, which generates an energetically favorable *trans*-amide linkage with weaker affinity for the active site. This allows the N-linked glycoprotein to dissociate from the OT complex and prevent potential product inhibition. An interesting possibility now exists to explain the presence of free oligosaccharides in the ER. If the native glycopeptide does not isomerize, the amide bond could be weakened, resulting in water-assisted hydrolysis to produce an oligosaccharide with a free reducing group. This could be mediated through unfavorable interactions between the oligosaccharide and the protein secondary structure [11], supporting the role for the primary amino acid sequence in determining glycosylation. Could this oligosaccharide be the source of the free $Glc_3Man_9GlcNAc_2$ in the ER lumen, and the reason why many consensus N-glycosylation sites are never occupied? Previous hypotheses have invoked a dolichyl oligosaccharide hydrolase activity of OT that is responsible for preferential transfer to water [12] under peptide acceptor reducing conditions. This would be an inefficient process and would not be regulated by protein determinants. However, if isomerization goes to completion and the product is released, the active site for transfer would now be accessible for dolichyl oligosaccharide binding to incoming peptide, ensuring a continuous and efficient supply of N-linked glycosylated protein, as specified by the protein sequence.

The Imperiali group also shows that the use of product mimics of OT reactants allows further definition of the mechanism of action of this enigmatic enzyme and demonstrates the exquisite means that enzymes use to control biologically significant processes in eukaryotic cells.

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Combinatorial Glycosylation of Glycopeptide Antibiotics

The glycosyltransferases GtfE and GtfD from the vancomycin producer *Amycolatopsis orientalis* have promiscuous substrate and NDP-sugar specificities. They have been used to generate novel glycopeptide antibiotics containing the heptapeptide scaffolds of vancomycin and teichoplanin [1].

The glycopeptide antibiotics vancomycin and teicoplanin are secondary metabolites produced by soil actinomycetes, *A. orientalis* and *Actinoplanes teichomyceticus,* respectively. Both are clinically important for the treatment of infections due to gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) [2, 3]. With the emergence of vancomycin-resistant enterococci (VRE) and *S. aureus* (VRSA), the development of new derivatives of vancomycin [4] and the development of other antibiotics with new modes of action (e.g., daptomycin [5]) are important for the treatment of life-threatening gram-positive infections.

Like vancomycin, chloroeremomycin is produced by a strain of *A. orientalis* [6]. Chloroeremomycin differs from vancomycin in its pattern of glycosylation. Vancomycin has the disaccacharide D-glucose-L-vancosamine attached to the phenolic group of hydroxyphenylglycine at amino acid 4 of the heptapeptide. Chloeremomycin has the disaccharide D-glucose-L-4-*epi*-vancosamine